

Figure S1A

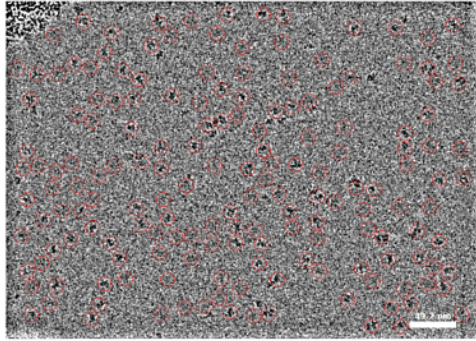


Figure S1B

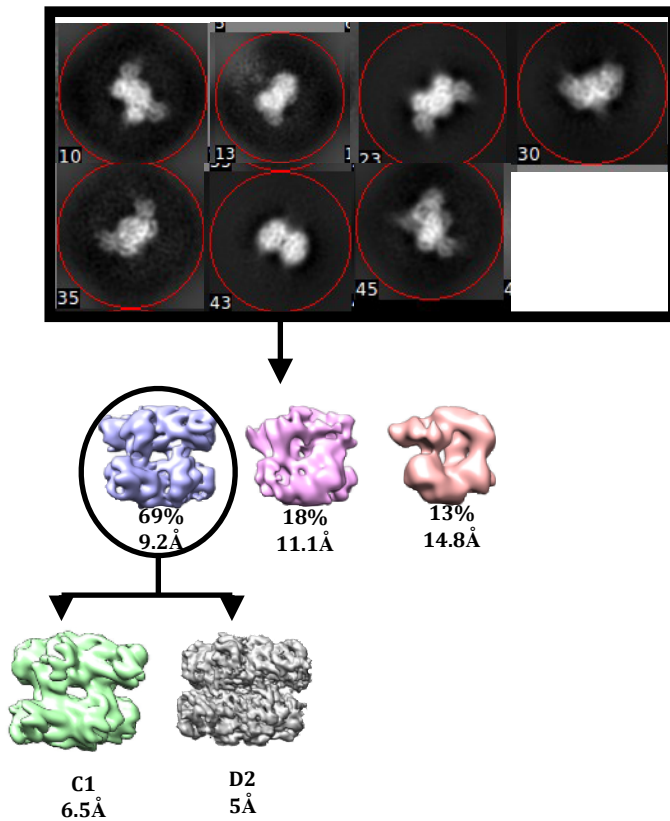


Figure S1A-E: CryoEM pipeline of CtBP2₃₁₋₃₆₄. (A) Raw images collecting on the Talos Artica. 485,473 particles were picked and subjected to reference-free 2D class averages. (B), (C), (D) represent different views and the final 3D reconstructions from those specific classes. (E) since all the reconstructions show a tetramer, all the classes were combined for the final reconstruction in (F). (F) Initial maps were generated in cisTEM, 3D classification, 3D refinement, CTF refinement and post-processing were done in Relion 3.0.

Figure S1C

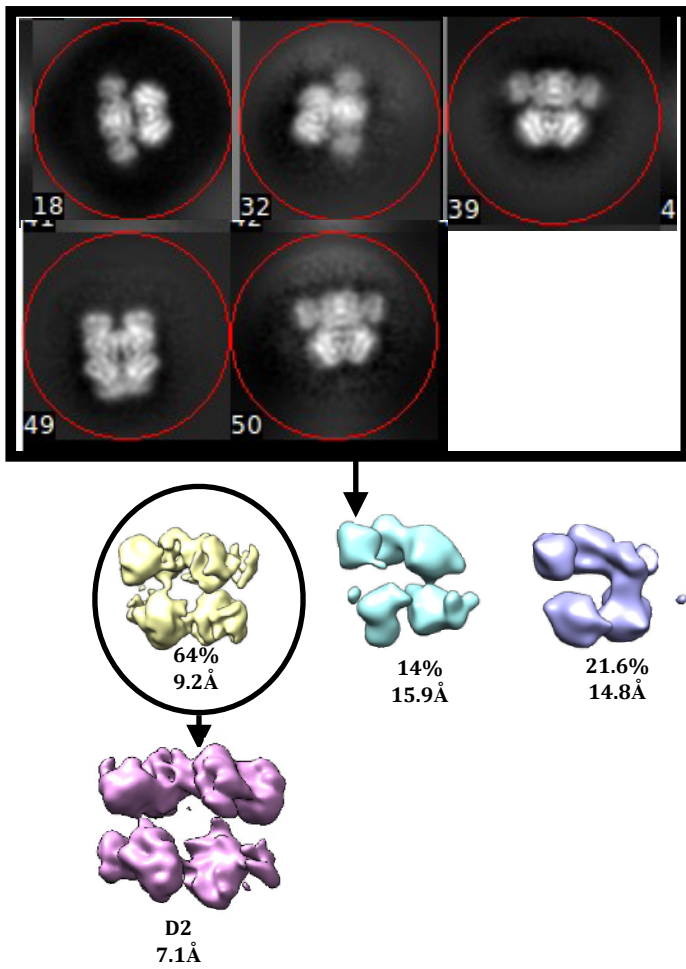


Figure S1D

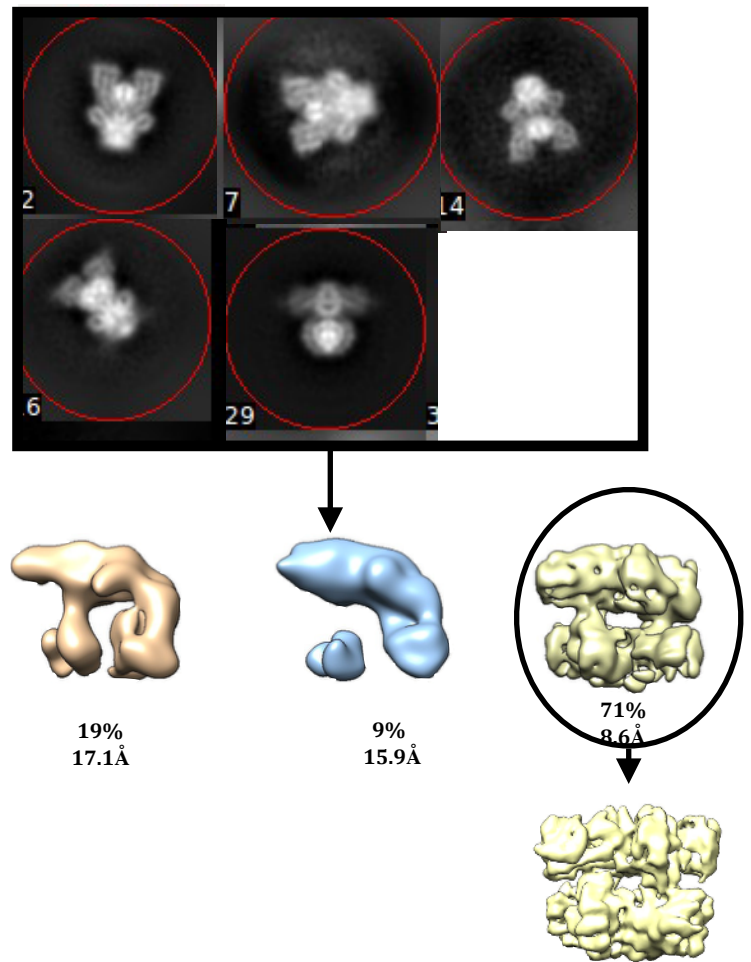


Figure S1E

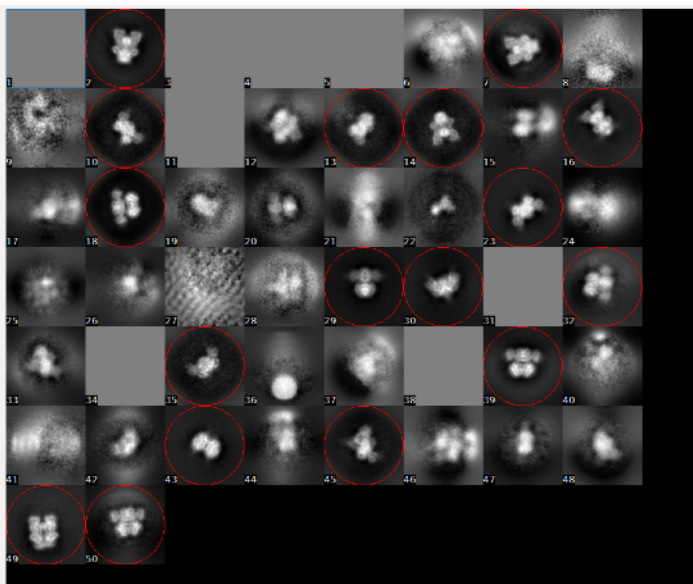


Figure S1F

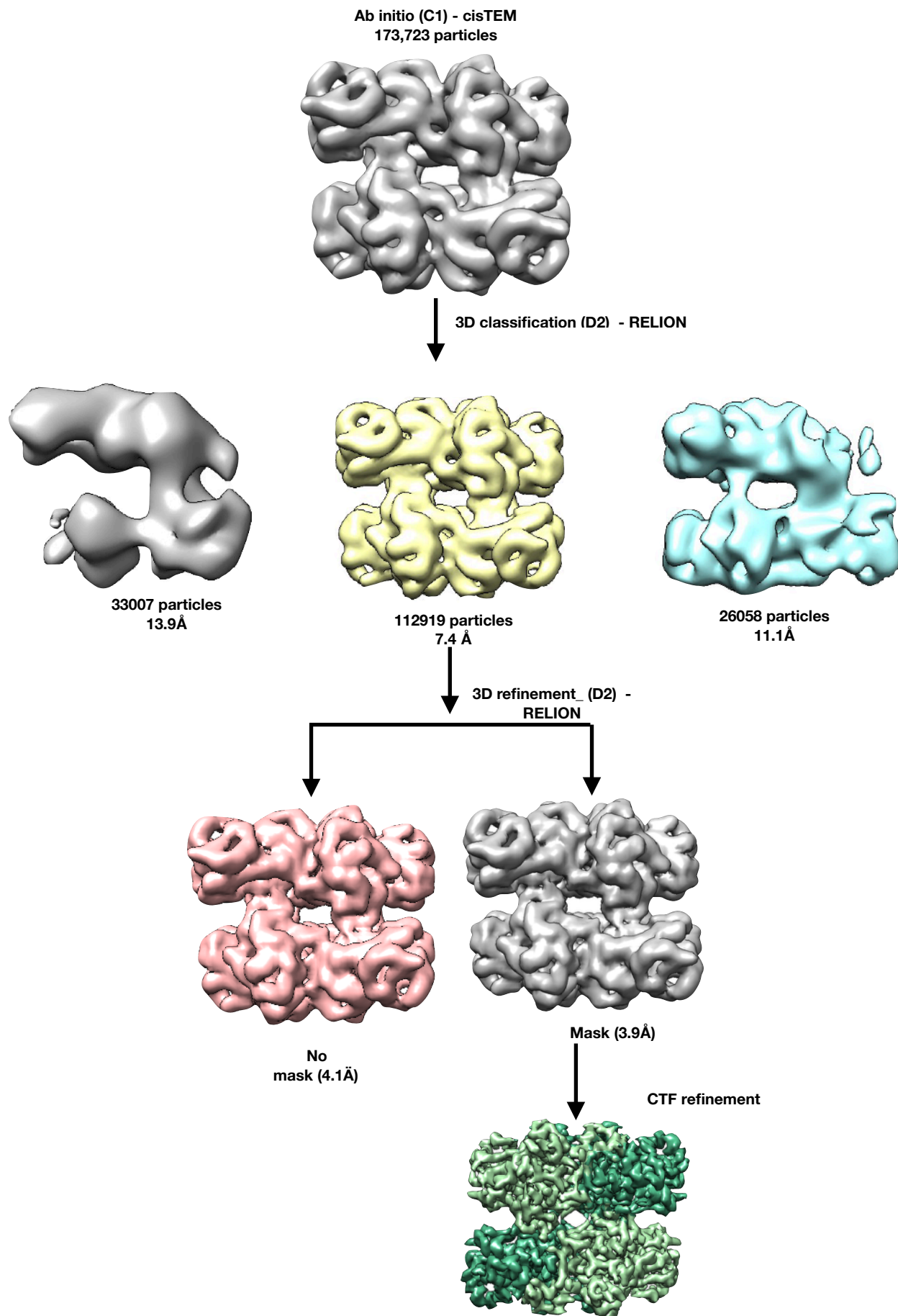


Figure S2A

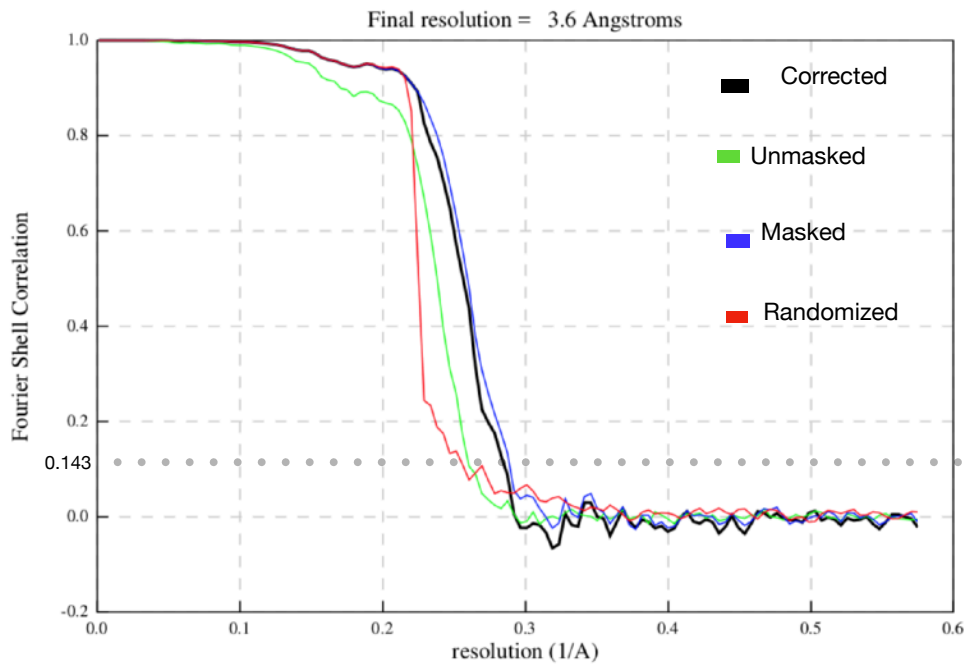


Figure S2B

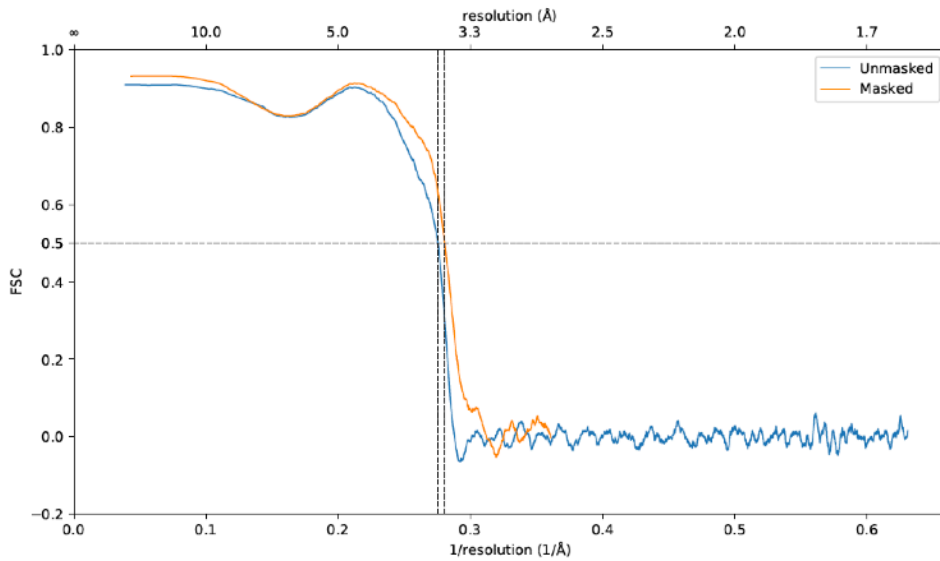


Figure S2C

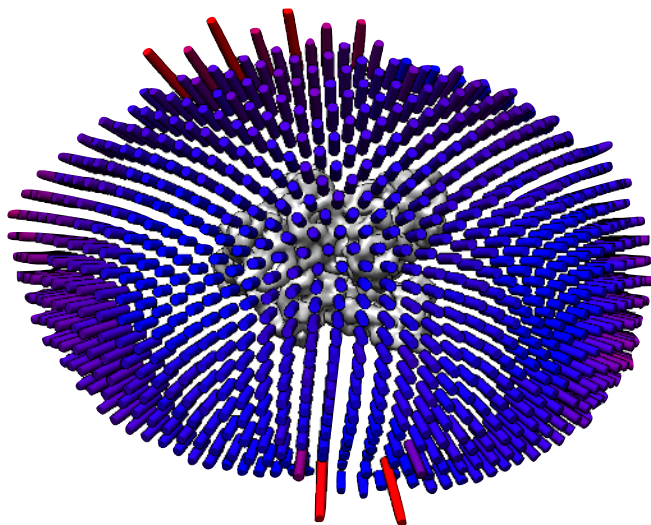


Figure S2- Map Resolution: (A) FSC curve for CtBP₂₃₁₋₃₆₄ reconstruction. (B) Map-to-model FSC showing agreement between the atomic model and experimental map. (C) Euler angle distribution of particles in final 3D reconstruction.

Figure S3A

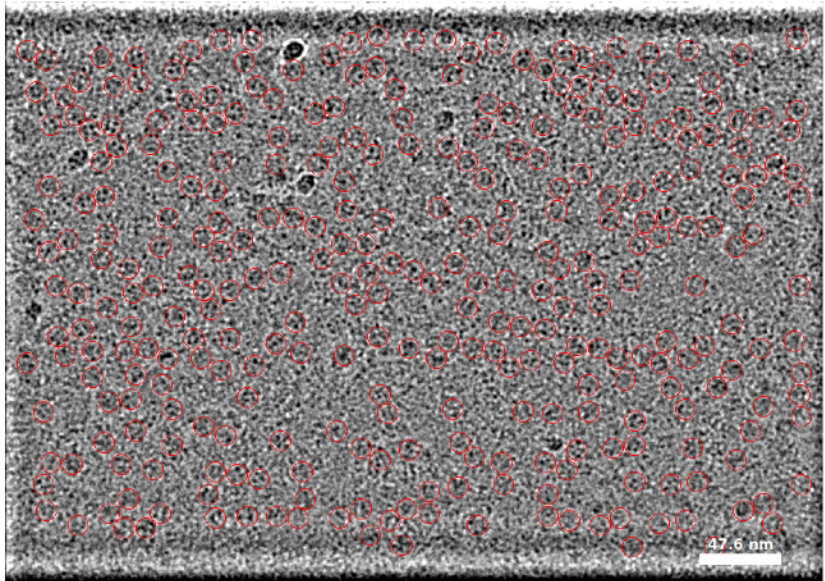
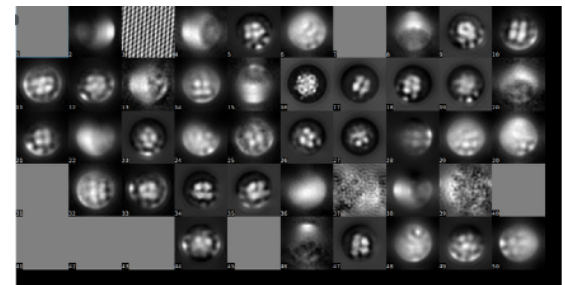
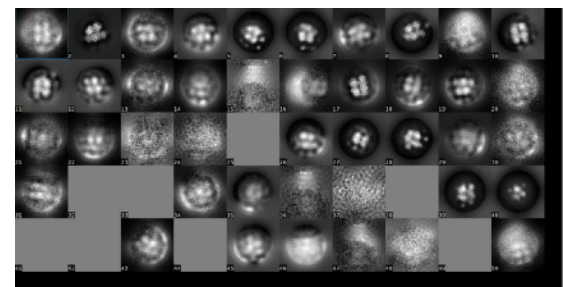


Figure S3B

Round 1



Round 2



Round 3

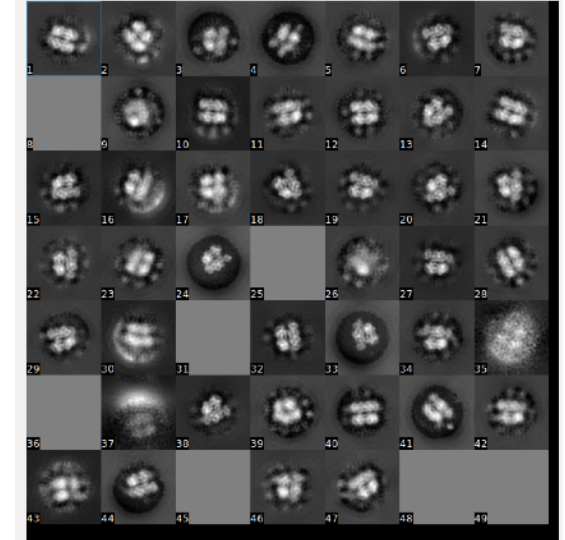


Figure S3C

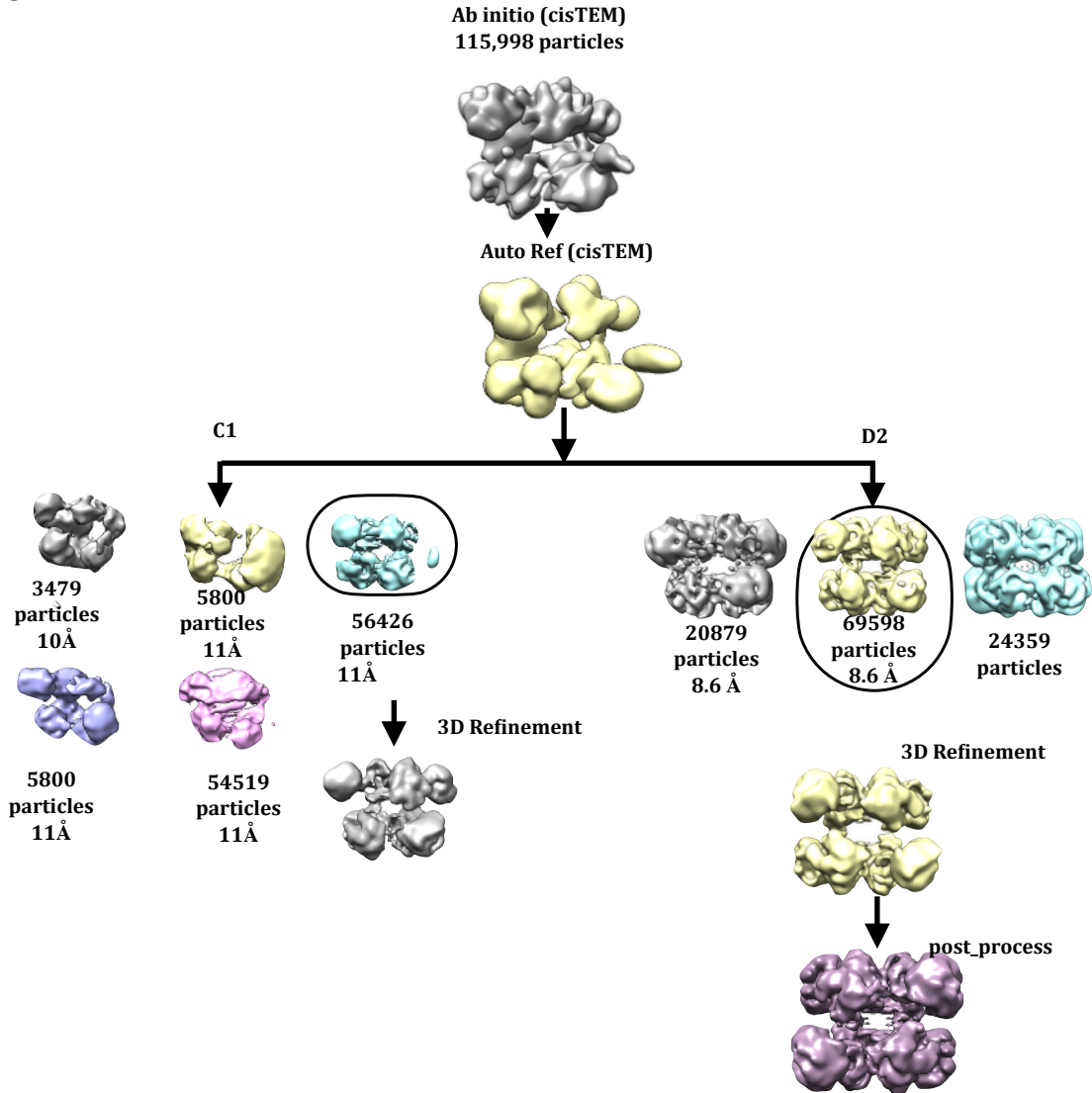


Figure S3D

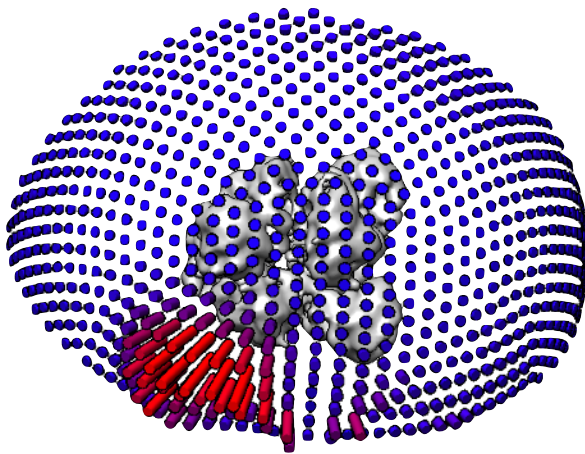


Figure S3_ CryoEM pipeline of CtBP2₃₁₋₄₄₅: (A) Raw micrograph of CtBP2₃₁₋₄₄₅. Particle picking was done in cisTEM. Red circles indicate picked particles. (B) Three round of 2D classification. (C) 3D reconstruction and refinement of CtBP2₃₁ in Relion. (D) Euler angle distribution of CtBP2₃₁₋₄₄₅ final reconstruction.

Figure S4- Orthogonal slices of the ab initio reconstruction of CtBP2₃₁₋₄₄₅ showing diffuse density indicated with red arrow.

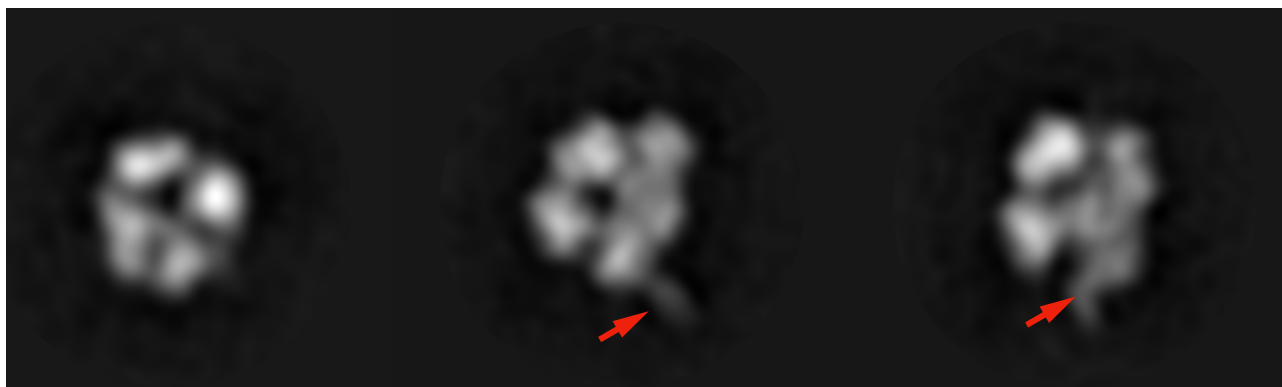


Figure S5- Side by side comparison of a low-pass filtered map of CtBP2₃₁₋₃₆₄ and CtBP2₃₁₋₄₄₅.

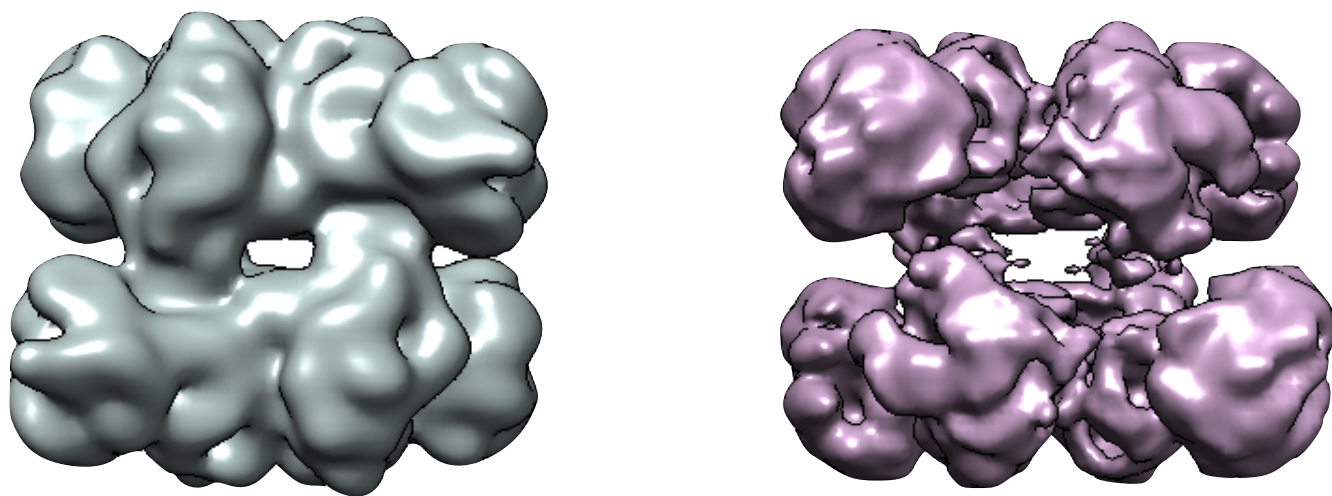


Figure S6

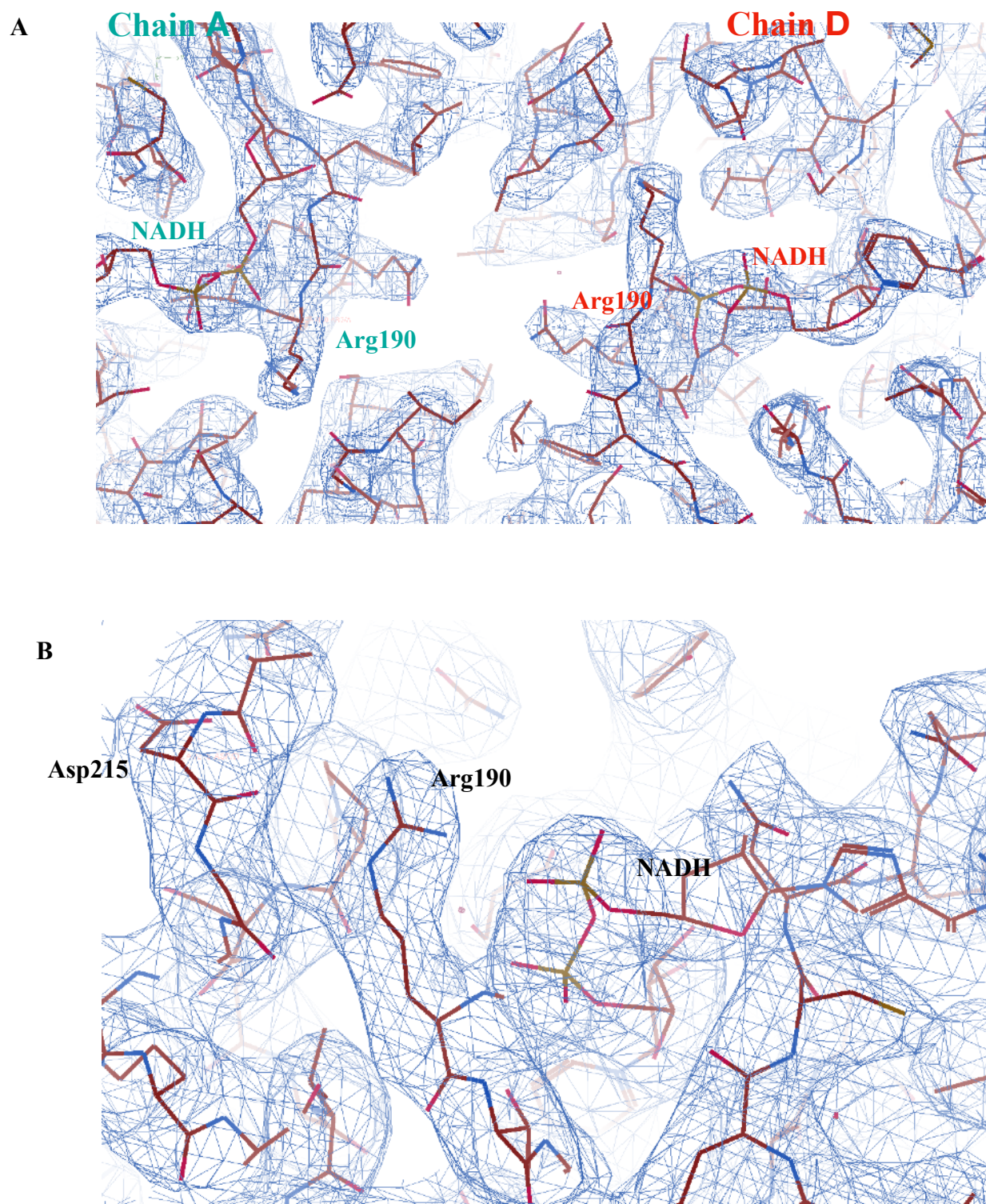


Figure S6- Representative density of dimer interface and interactions.

Figure S7

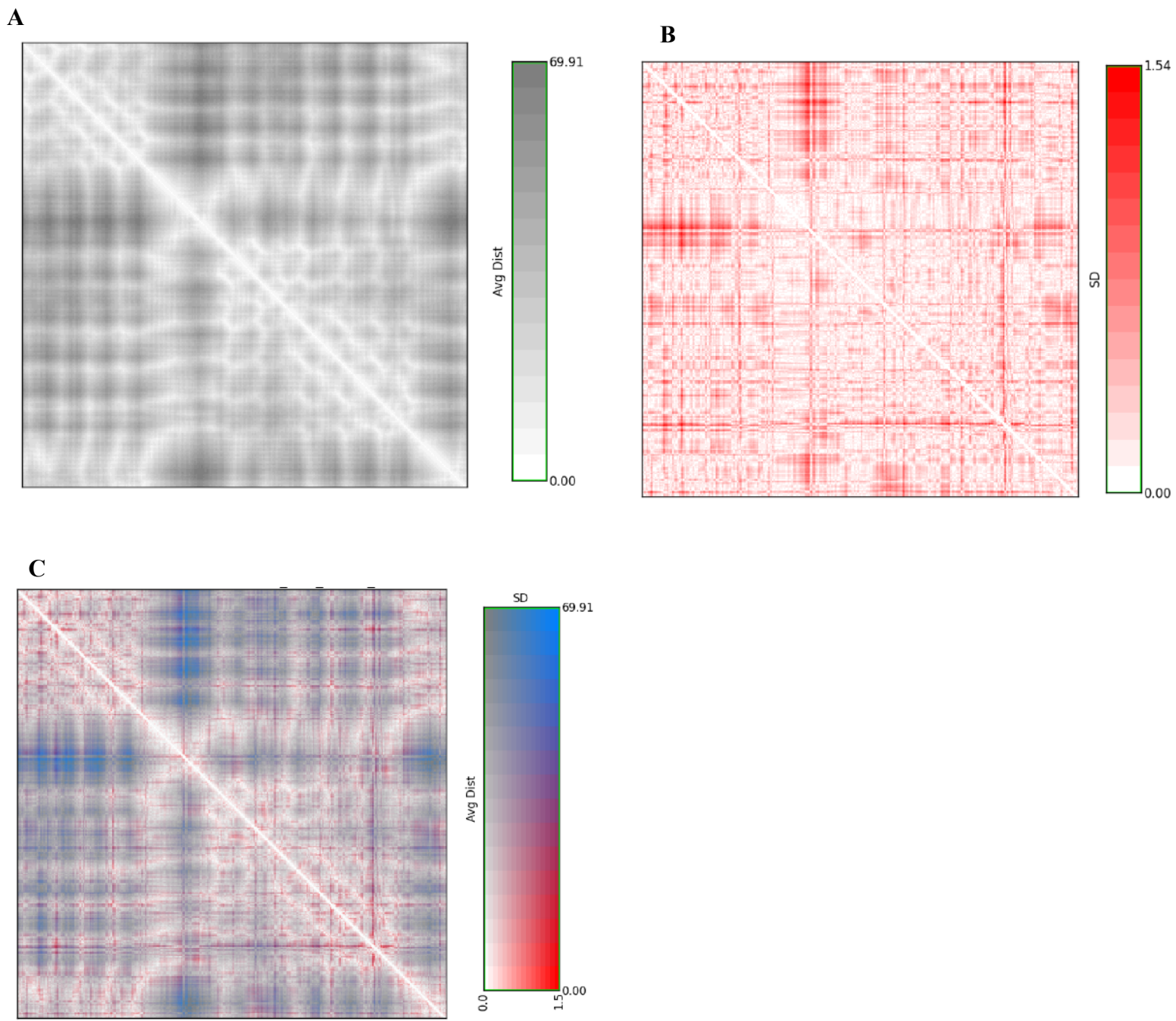


Figure S7- Ca-Ca distance map analysis of cryoEM and Crystallography structures. (A) Average distance in Å . (B) Standard deviation in Å. (C) Average distance (Y-axis) and Standard deviation (X-axis) plotted on the same 2D matrix. Distances and deviations calculations were done with residues from all chains.

Figure S8

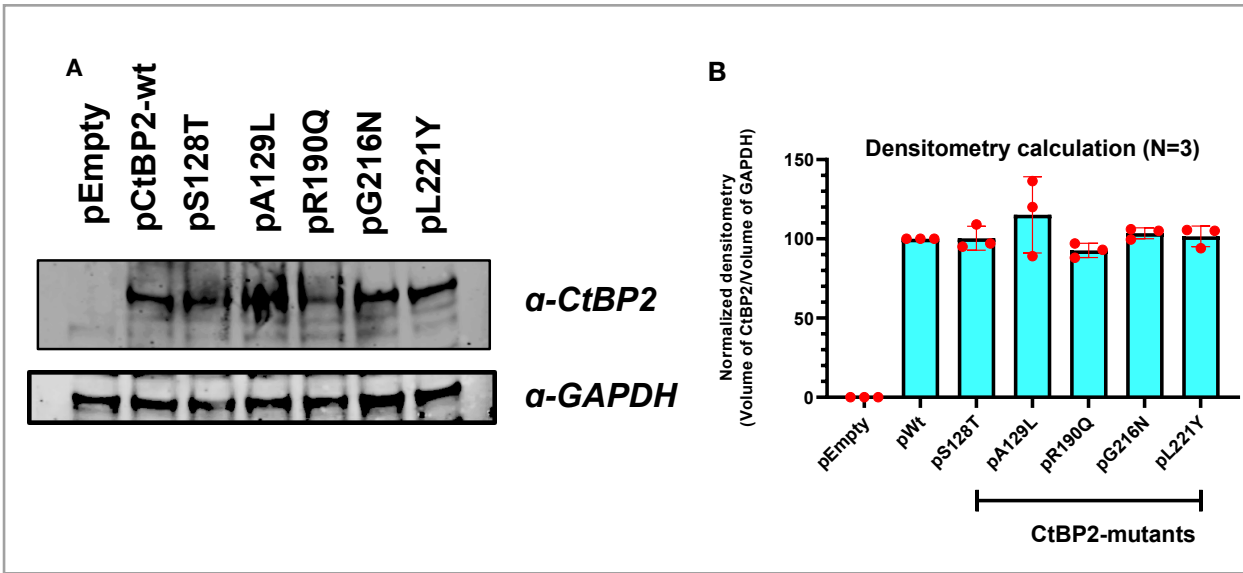


Figure S8- Expression level of CtBP2 variants. (A) Representative CtBP2 immunoblot of lysates from transfected HCT116; CtBP2(-/-) cells. (B) Mean CtBP2 and CtBP2 variant abundance derived by densitometric quantification of CtBP2 immunoblots performed on transfected cell lysates from each replicate. N=3. Red circles indicate individual values from each replicate, and error bars indicate ± 1 standard deviation.